SECONDARY PROTHALLIA OF *NEPHRODIUM HIRTIPES* HK

By W. N. Steil

Although the prothallia of a large number of species of ferns were grown under different cultural conditions to determine, so far as possible, what factors are concerned in the formation of secondary prothallia, the results herein described are based chiefly on experiments with the prothallia of *Nephrodium hirtipes* for a description of which the reader is referred to an earlier paper (Steil, 1919).

In the first culture of *Nephrodium hirtipes*, made December 14, 1913, most of the prothallia were destroyed by a parasitic fungus. The healthy prothallia were carefully transferred to cultures made by placing sphagnum into small Stender dishes. The sphagnum was saturated with Knop's solution, and the medium was then thoroughly sterilized before the prothallia were transplanted. The cultures were then placed in subdued light for a period of two weeks. In consequence of the different light conditions, a large number of short filaments, each consisting of a single row of cells, were produced from the margins and occasionally from both surfaces of the prothallia. The cultures were then placed for a like period of time under favorable conditions of illumination in a Wardian case. It was now observed that the filamentous prothallia broadened out and became heart-shaped. The experiment was repeated several times with the same culture and as a result of the changes in illumination numerous secondary prothallia were obtained which upon separation from one another became independent prothallia (Fig. 5, Plate XXVI). Fig. 1, Plate XXV represents a large secondary prothallium obtained from one of the cultures. From a margin of the prothallium a lobe (a) has been produced. A smaller heart-shaped prothallium (c) has been formed as an outgrowth of the lobe. The posterior portion of the large prothallium consists almost wholly of dead cells. The prothallium bears an apogamously produced embryo (e) of considerable size. An embryo of like origin had just begun its development on the

smaller prothallium. The secondary prothallia which were produced after the primary prothallia were transferred from subdued light to favorable illumination resembled those represented in Fig. 6, Plate XXVI. These, however, as will be described later, were produced under different cultural conditions.

A culture of *Polypodium crassifolium* L. was placed under the same conditions of illumination as those under which the culture of *Nephrodium hirtipes* produced numerous secondary prothallia and it was found that the former species exhibited a similar tendency to form such prothallia. It thus appears that, although the prothallia of *Nephrodium hirtipes* produce embryos apogamously, they possess no greater tendency to vegetative growth than do those of *Polypodium crassifolium* which produce embryos only as a result of fertilization.

In many cultures of *Nephrodium hirtipes* and several other species of ferns, the method just described for producing secondary prothallia was invariably successful.

Large portions of prothallia were removed from cultures in the Wardian case and were placed on media like that of the cultures just described. Some pieces were also floated on the surface of sterilized tap water, and sterilized nutrient solutions. Even when the illumination was favorable for the formation of heart-shaped prothallia in cultures made by sowing the spores, numerous secondary prothallia were produced from the margins and surfaces of the larger pieces (Fig. 2, Plate XXV).

When prothallia, growing under favorable light conditions, were cut off near the substratum with a sharp razor, the remaining portions of the prothallia likewise produced many secondary prothallia (Steil, 1918).

In some of the cultures the prothallia were attacked by parasitic fungi to such an extent that only small portions of the older prothallia remained. From the apparently normal cells of such prothallia, filaments were produced which developed into heart-shaped prothallia when the cultural conditions became more favorable.

Occasionally the prothallia of *Nephrodium hirtipes*, especially in the older cultures, became discolored or brownish, perhaps on account of certain "physiological" conditions. The large majority of the cells in such cases died. From the living cells secondary prothallia were usually formed when more nutrient solution was supplied

to the culture. The writer has observed in the vicinity of Madison similar instances of regeneration of the prothallia of *Onoclea sensibilis* L. which had lived over winter. In some cases only small portions of the original prothallia had survived the winter conditions. From these portions secondary prothallia were observed to form in profusion.

When the illumination was somewhat subdued, but not sufficiently to produce only filamentous prothallia, one or more lobes of the primary prothallium formed secondary prothallia (Fig. 3, Plate XXV, and Fig. 6, Plate XXVI).

Prothallia of *Nephrodium hirtipes* on which apogamous embryos had begun their development were placed under conditions of weak illumination and these also formed secondary prothallia (Fig. 2 Plate XXV). The two regions, a and b, shown in the photograph, are composed of cells containing few chloroplasts. Embryos of apogamous origin have already begun their development in the paler regions.

Under certain conditions of light to be described at a later time, many branched cells were produced (Fig. 7, Plate XXIV). When the cultures were placed in the Wardian case, the branched cells formed prothallia precisely like those originating from the germination of a spore (Fig. 8, Plate XXIV). Branched cells have been described by Atkinson (1894), Miss Black (1915) and Miss Wuist (1916). Atkinson (1894) reported the formation of prothallia from branched cells of *Adiantum cuneatum*.

In one of the cultures of *Nephrodium hirtipes*, a peculiar secondary prothallium was observed (Fig. 4, Plate XXV). The "light" region present in the portion just back of the apical notch indicates that an embryo of apogamous origin was about to make its appearance. A number of clearly defined regions are shown at *a*, Fig. 4. At *b* is a larger and more distinct area. All of those at *a* in a few days fromed small prothallia. The prothallium was composed of only a single layer of cells in thickness where the peculiar regions were present. The writer is unable to give a satisfactory explanation the prothallium described above.

Secondary prothallia were readily induced by any of the methods which have been described. In every respect, such prothallia

resembled apparently the primary ones, producing also embryos of apogamous origin.

The formation of secondary prothallia from primary prothallia have been described by a large number of investigators including, Wiegand (1849), Hofmeister (1851), Kny (1870), Goebel (1877), de Bary (1878), Bauke (1878), Beck (1880), Dodel-Port (1880), Campbell (1892), Heim (1896), Britton and Taylor (1902), Lagerberg (1906), Woronin (1908), Pace (1913), Heilbron (1910), Fischer (1911), Schlumberger (1911), Wuist (1913), Nagai (1914), Pickett (1914), Black (1914), Wuist (1916).

Department of Botany.
University of Wisconsin.

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DESCRIPTION OF PLATES

Plate XXV

The prothallia from which the photo-micrographs 1, 2, and 3 were made, were magnified about 25 times. Photomicrograph 4 represents a magnification of about 30 times.

Fig. 1. A prothallium of *Nephrodium hirtipes*. A large lobe, *a*, has been produced from the prothallium. From the lobe a smaller heart-shaped prothallium, *c*, has been formed. Apogamous embryo, *e*.

Fig. 2. A prothallium of *Nephrodium hirtipes* from which many secondary prothallia have been formed. Regions in which apogamous embryos are beginning their development, *a* and *b*.

Fig. 3. A prothallium of *Pteris cretica albo-lineata* from one lobe of which regeneration has taken place. An embryo of apogamous origin has also begun its development at *a*.

Fig. 4. A peculiar secondary prothallium of *Nephrodium hirtipes*. Distinct prothallial regions at *a* and *b*.

Plate XXVI

Fig. 5. A culture of *Nephrodium hirtipes* containing numerous secondary prothallia. X1¼

Fig. 6. Prothallia of *Nephrodium hirtipes* from the lobes of which secondary prothallia have been produced. X 2½.

Fig. 7. Branched cells of *Nephrodium hirtipes*. X About 42.

Fig. 8. A young prothallium of *Nephrodium hirtipes* produced from a branched cell. X 42.